WILEY
Online Proofing System Instructions

The Wiley Online Proofing System allows proof reviewers to review PDF proofs, mark corrections, respond to queries, upload replacement figures, and submit these changes directly from the locally saved PDF proof.

1. For the best experience reviewing your proof in the Wiley Online Proofing System ensure you are connected to the internet. This will allow the PDF proof to connect to the central Wiley Online Proofing System server. If you are connected to the Wiley Online Proofing System server you should see a green check mark icon above in the yellow banner.


Connected


Disconnected

2. Please review the article proof on the following pages and mark any corrections, changes, and query responses using the Annotation Tools outlined on the next 2 pages.

3. Save your proof corrections by clicking the "Publish Comments" button in the yellow banner above. Corrections don't have to be marked in one sitting. You can publish comments and log back in at a later time to add and publish more comments before you click the "Complete Proof Review" button below.

4. If you need to supply additional or replacement files bigger than 5 Megabytes (MB) do not attach them directly to the PDF Proof, please click the "Upload Files" button to upload files:

Click Here

5. When your proof review is complete and all corrections have been published to the server by clicking the "Publish Comments" button, please click the "Complete Proof Review" button below:

IMPORTANT: Did you reply to all author queries found on the first page of your proof?

IMPORTANT: Did you click the "Publish Comments" button to save all your corrections? Any unpublished comments will be lost.

IMPORTANT: Once you click "Complete Proof Review" you will not be able to add or publish additional corrections.

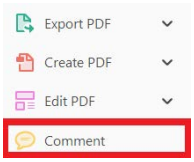
Click Here

WILEY

USING e-ANNOTATION TOOLS FOR ELECTRONIC PROOF CORRECTION

Required software to e-Annotate PDFs: Adobe Acrobat Professional or Adobe Reader (version 11 or above). (Note that this document uses screenshots from Adobe Reader DC.)
The latest version of Acrobat Reader can be downloaded for free at: http://get.adobe.com/reader/

Once you have Acrobat Reader open on your computer, click on the Comment tab (right-hand panel or under the Tools menu).



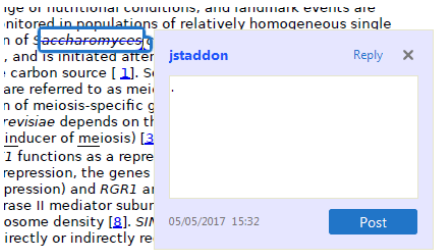
This will open up a ribbon panel at the top of the document. Using a tool will place a comment in the right-hand panel. The tools you will use for annotating your proof are shown below:

1. Replace (Ins) Tool – for replacing text.

Strikes a line through text and opens up a text box where replacement text can be entered.

How to use it:

- Highlight a word or sentence.
- Click on .
- Type the replacement text into the blue box that appears.

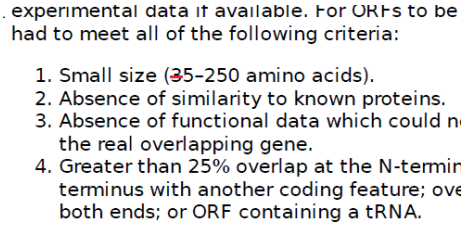


2. Strikethrough (Del) Tool – for deleting text.

Strikes a red line through text that is to be deleted.

How to use it:

- Highlight a word or sentence.
- Click on .
- The text will be struck out in red.

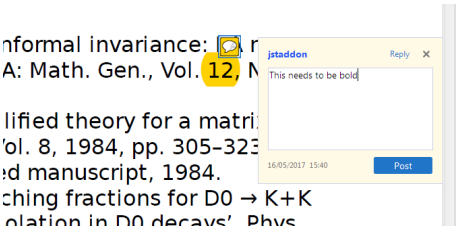


3. Commenting Tool – for highlighting a section to be changed to bold or italic or for general comments.

Use these 2 tools to highlight the text where a comment is then made.

How to use it:

- Click on .
- Click and drag over the text you need to highlight for the comment you will add.
- Click on .
- Click close to the text you just highlighted.
- Type any instructions regarding the text to be altered into the box that appears.

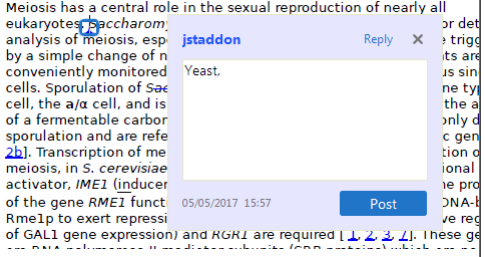


4. Insert Tool – for inserting missing text at specific points in the text.

Marks an insertion point in the text and opens up a text box where comments can be entered.

How to use it:

- Click on .
- Click at the point in the proof where the comment should be inserted.
- Type the comment into the box that appears.



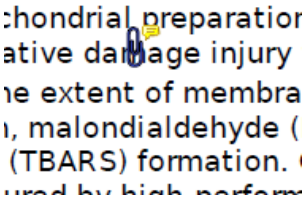
5. Attach File Tool – for inserting large amounts of text or replacement figures.

Inserts an icon linking to the attached file in the appropriate place in the text.

How to use it:

- Click on  .
- Click on the proof to where you'd like the attached file to be linked.
- Select the file to be attached from your computer or network.
- Select the colour and type of icon that will appear in the proof. Click OK.

The attachment appears in the right-hand panel.

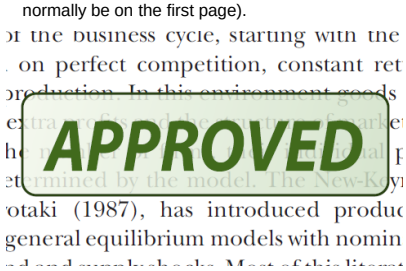


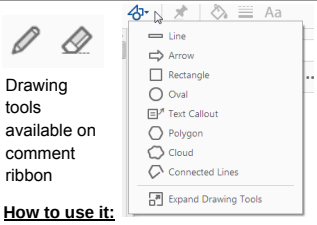
6. Add stamp Tool – for approving a proof if no corrections are required.

Inserts a selected stamp onto an appropriate place in the proof.

How to use it:

- Click on  .
- Select the stamp you want to use. (The Approved stamp is usually available directly in the menu that appears. Others are shown under *Dynamic*, *Sign Here*, *Standard Business*).
- Fill in any details and then click on the proof where you'd like the stamp to appear. (Where a proof is to be approved as it is, this would normally be on the first page).

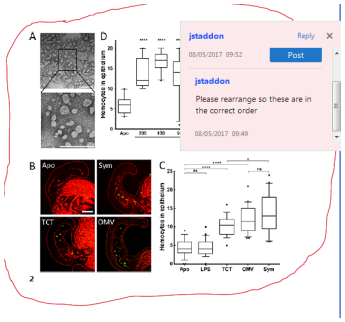




Drawing tools available on comment ribbon

7. Drawing Markups Tools – for drawing shapes, lines, and freeform annotations on proofs and commenting on these marks.

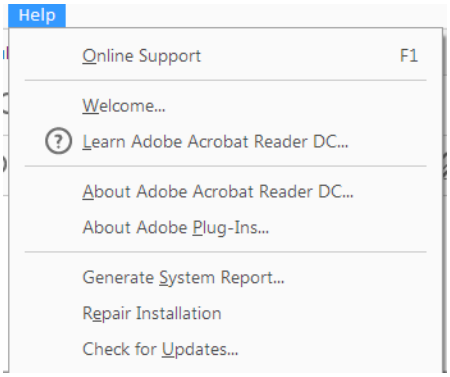
Allows shapes, lines, and freeform annotations to be drawn on proofs and for comments to be made on these marks.



How to use it:

- Click on one of the shapes in the Drawing Markups section.
- Click on the proof at the relevant point and draw the selected shape with the cursor.
- To add a comment to the drawn shape, right-click on shape and select *Open Pop-up Note*.
- Type any text in the red box that appears.

For further information on how to annotate proofs, click on the Help menu to reveal a list of further options:



WILEY

Author Query Form

Journal: JEB

Article: 13219

Dear Author,

During the copyediting of your manuscript the following queries arose.

Please refer to the query reference callout numbers in the page proofs and respond to each by marking the necessary comments using the PDF annotation tools.

Please remember illegible or unclear comments and corrections may delay publication.

Many thanks for your assistance.

Query reference	Query	Remarks
1	AUTHOR: A running head short title was not supplied; please check if this one is suitable and, if not, please supply a short title of up to 40 characters that can be used instead.	OK
2	AUTHOR: Please verify that the linked ORCID identifiers are correct for the authors "G. D. Cunningham and E. Wapstra".	see note
3	AUTHOR: Please confirm that given names (red) and surnames/family names (green) have been identified correctly.	OK
4	AUTHOR: Please provide fax number for corresponding author.	N/A
5	AUTHOR: Jones *et al.*, 1997 has not been included in the Reference List, please supply full publication details.	see note
6	AUTHOR: Australian Government Bureau of Meteorology, 2015 has not been included in the Reference List, please supply full publication details.	see note
7	AUTHOR: Cadby et al. (2011) has not been cited in the text. Please indicate where it should be cited; or delete from the Reference List.	deleted
8	AUTHOR: Uller and While (2014) has not been cited in the text. Please indicate where it should be cited; or delete from the Reference List.	deleted

Funding Info Query Form

Please confirm that the funding sponsor list below was correctly extracted from your article: that it includes all funders and that the text has been matched to the correct FundRef Registry organization names. If a name was not found in the FundRef registry, it may not be the canonical name form, it may be a program name rather than an organization name, or it may be an organization not yet included in FundRef Registry. If you know of another name form or a parent organization name for a "not found" item on this list below, please share that information.

FundRef name	**FundRef Organization Name**
Holsworth Wildlife Research Fund	Holsworth Wildlife Research Endowment
Foundation of National Parks	N/A

J E B	13219	WILEY	Dispatch: 7.12.17	CE: Lenard S
Journal Code	Manuscript No.		No. of pages: 10	PE: Bhagyalakshmi

doi: 10.1111/jeb.13219

Disentangling sex allocation in a viviparous reptile with temperature-dependent sex determination: a multifactorial approach

J. GRUBER*[1], G. D. CUNNINGHAM†[1], G. M. WHILE† & E. WAPSTRA†

*School of Biological Sciences, The University of Sydney, Sydney, NSW, Australia
†School of Biological Sciences, The University of Tasmania, Hobart, Tas., Australia

Keywords:

maternal allocation;
reptile;
sex ratio;
squamate;
temperature-dependent sex determination.

Abstract

Females are predicted to alter sex allocation when ecological, physiological and behavioural variables have different consequences on the fitness of male and female offspring. Traditionally, tests of sex allocation have examined single causative factors, often ignoring possible interactions between multiple factors. Here, we used a multifactorial approach to examine sex allocation in the viviparous skink, *Niveoscincus ocellatus*. We integrated a 16-year observational field study with a manipulative laboratory experiment to explore whether the effects of the maternal thermal environment interact with the resources available to females for reproduction to affect sex allocation decisions. We found strong effects of temperature on sex allocation in the field, with females born in warm conditions and males in cold conditions; however, this was not replicated in the laboratory. In contrast, we found no effect of female resource availability on sex allocation, either independently, or in interaction with temperature. These results corresponded with an overall lack of an effect of resource availability on any of the life history traits that we predicted would mediate the benefits of differential sex allocation in this system, suggesting that selection for sex allocation in response to resource availability may be relatively weak. Combined, these results suggest that temperature may be the predominant factor driving sex allocation in this system.

Introduction

How a female divides her reproductive energy among male and female offspring is the key question underpinning sex allocation theory (Charnov, 1982; West, 2009; Wapstra & Warner, 2010). An unequal allocation of resources to male vs. female offspring is expected either when extrinsic or intrinsic factors provide sex-specific effects on offspring fitness, or when the costs of producing male or female offspring differ (Fisher, 1930; Charnov, 1982). Significant sex allocation biases are not uncommon in nature, having been found in a wide range of taxa, including haplodiploid insects (Charnov, 1979), fish (Conover, 1984), lizards (Warner & Shine, 2008), birds (Komdeur, 1996) and mammals (Cameron & Dalerum, 2009). A major challenge for evolutionary biologists is to understand the factors that drive or constrain differential allocation of maternal resources between the sexes.

To address this challenge, empirical and theoretical biologists have attempted to explain how various factors, including the environment, demography, physiology and behaviour influence sex allocation patterns (Trivers & Willard, 1973; West, 2009). However, although these models have proved successful in predicting sex allocation in some systems (especially invertebrates; Seger & Stubblefield, 2002), the field is plagued by a lack of consistency in observed patterns of sex allocation among studies and limited agreement between theoretical predictions and empirical patterns (Frank, 1990; Wapstra & Warner, 2010). These inconsistencies may arise for several reasons. For example,

Correspondence: Erik Wapstra, School of Biological Sciences, University of Tasmania, Hobart, Tas. 7001, Australia.
Tel.: +61 427 093 693; fax: xxxx; e-mail: erik.wapstra@utas.edu.au
[1]Equal first author.

the majority of sex allocation hypotheses focus on a single factor, ignoring the possibility that the multifactorial complexities of life histories in natural systems may obscure results if multiple factors interact (Cockburn *et al.*, 2002; Seger & Stubblefield, 2002). Second, the integration of observational and experimental studies is rare. This is problematic, as focussing solely on laboratory results may reveal sex allocation responses that are artefacts of an experimental environment (Frank, 1990; Komdeur & Pen, 2002) although observational studies provide only correlational evidence, and may misattribute the true causes of observed patterns.

In this study, we applied a multifactorial approach to examine sex allocation in the viviparous skink, *Niveoscincus ocellatus* (Gray, 1845). We have previously demonstrated that offspring sex is affected by temperature in this species (Wapstra *et al.*, 2004, 2009; Pen *et al.*, 2010; Cunningham *et al.*, 2017). In *N. ocellatus*, as in other ectotherms, warmer conditions result in earlier dates of birth because of accelerated embryonic development. This, in turn, results in a longer growth period in the first year of life, which enhances female, but not male, fitness (Wapstra, 2000; Pen *et al.*, 2010; Wapstra *et al.*, 2010). Coupling offspring sex to temperature, then, provides a mechanism by which mothers maximize their fitness by skewing the sex ratio of litters towards females when high temperatures lead to early births, and towards males when low temperatures lead to later births (Pen *et al.*, 2010). Unlike other reptilian taxa in which offspring sex is affected by temperature (e.g. turtles and crocodiles), where the reaction norm of temperature effects on offspring sex is steep (i.e. 100% of each sex produced either side of a pivotal temperature), the reaction norm in *N. ocellatus* is shallow (Cunningham *et al.*, 2017). Furthermore, temperature alone does not account for the full variability in sex allocation patterns observed, suggesting that other factors may also affect these patterns. In other reptile species (Radder *et al.*, 2009), including the closely related snow skink, *Niveoscincus microlepidotus* (Olsson & Shine, 2001), offspring size at birth has sex-specific fitness effects, with females benefitting more than males from being born large. Females that produce larger offspring may therefore also skew sex allocation towards females (a reverse of predictions made under classic Trivers & Willard (1973)). Thus, any factor that affects the ability of females to manipulate offspring size may also influence sex allocation decisions in addition to, or in interaction with, temperature.

In *N. ocellatus*, energetic allocation to offspring occurs in two key periods; prior to ovulation, during an extended period of vitellogenesis from soon after the previous birth using capital and income energy sources (Wapstra *et al.*, 1999) and throughout gestation via a complex chorioallantoic placenta (i.e. matrotrophy; Thompson *et al.*, 2001). *Niveoscincus* is one of only five lineages of squamate reptiles in which substantial placentotrophy occurs. Indeed, in *N. ocellatus*, neonates are more than 1.5 times larger in dry matter than freshly ovulated eggs (Thompson *et al.*, 2001). Thus, offspring size at birth may be affected by resources available to females both prior to ovulation and during gestation. Resources available during either of these stages may therefore influence sex allocation decisions. Importantly, such effects may interact with the known effects of temperature. For instance, female basking decisions may be altered by body condition (e.g. females in better condition may be able to devote more time to basking). Conversely, nutritional and energetic requirements of offspring may be influenced by temperature (as, in ectotherms, metabolism is temperature-dependent) or the process of nutrient assimilation across the placenta may itself be temperature-dependent (Wapstra, 2000).

To explore the potential effect of resource availability on sex allocation decisions in *N. ocellatus*, we combined a manipulative laboratory experiment with data from a 16-year observational field study. We predicted that females producing larger offspring would overproduce daughters (and vice versa) and that, in line with previous results, females would produce female-skewed litters in warm years (field study) or when given access to increased basking opportunities (laboratory study) and male-skewed litters in cold years, or when given reduced basking opportunities. We also predicted that females in better condition at the beginning of vitellogenesis (field study) or at ovulation (laboratory study) would increase their reproductive effort. If this resulted in larger offspring (rather than increased number of offspring), then we predicted that these offspring would also be more likely to be female. In *N. ocellatus*, litter size is determined during vitellogenesis and is fixed at ovulation. We therefore predicted that additional resources provided to females after this time (i.e. during gestation; laboratory study) would lead to an increase in offspring size, and female-skewed litters. Because of the potential for temperature and resource availability to alter basking or investment decisions and therefore to have interacting effects on offspring size and dates of birth (and thus offspring sex), we also considered interactions between resource availability and temperature or basking in our analyses. Specifically, we predicted that when these factors acted in the same direction (i.e. low temperature and low resource availability would lead to male offspring or high temperature and high resource availability would lead to female offspring), the effects of the factors would reinforce each other. Conversely, when factors acted in different directions (i.e. low temperatures and high resource availability or high temperatures and low resource availability), effects on offspring sex would counteract each other and the overall strength and direction of the response would depend on the competing magnitude of these effects.

Materials and methods

Study species

Niveoscincus ocellatus is a small viviparous skink (3–10 g, 60–75 mm snout-vent length (SVL)) endemic to Tasmania, Australia. For the past 16 years (since the austral summer of 2000/2001), a population located on the East Coast (42°55′S, 147°87′E) has been monitored, following a standard field and laboratory protocol (see Wapstra & O'Reilly, 2001; Wapstra *et al.*, 2009; Cunningham *et al.*, 2017). The study site is flanked on all sides by either roads or unsuitable habitat and, consequently, there is little immigration or emigration. At this site, animals are active from August through May and females reproduce annually (Wapstra *et al.*, 1999). Mating occurs predominately in the Austral autumn (March to May), with a second mating period in spring (late August to September), and females store sperm throughout winter (Jones *et al.*, 1997; While & Wapstra, 2009). Vitellogenesis commences soon after parturition (Wapstra *et al.*, 1999) and ovulation is highly synchronized within the population, occurring in early October each year (Wapstra *et al.*, 1999, 2009; Pen *et al.*, 2010; Uller *et al.*, 2011). Gestation length is variable among years, with births occurring earlier in warmer years and later in cooler years (Wapstra *et al.*, 2009; Cadby *et al.*, 2010; Uller *et al.*, 2011; Cunningham *et al.*, 2017). Females produce clutches of one to six offspring annually (although litters larger than four are rare), and there is no post-natal care (Wapstra *et al.*, 1999; Wapstra & Swain, 2001). Individuals reach maturity at 2–3 years of age and can live for more than 7 years (Wapstra *et al.*, 2001; Pen *et al.*, 2010).

Field study: animal capture and data collection

Each year pregnant females (up to 100 females per year, representing 90–95% of the adult female population) were caught towards the end of gestation in late December (well after the period of sex determination; Neaves *et al.*, 2006; Wapstra *et al.*, 2009). Upon capture, females were identified using unique toe clips and their capture location was recorded before they were brought to a temperature-controlled laboratory (ambient temperature 16 °C) at the University of Tasmania. Females were then weighed (±1 mg) and transferred to individual plastic terraria (30 × 20 × 20 cm), each containing paper pellets as a substrate, a wooden cover for shelter and a rock for basking. A basking light was fitted over each terrarium to provide a thermal gradient for basking from 16 to 40 °C, and water was available *ad libitum*. Three times per week, all lizards were fed live insects (*Tenebrio* larvae) and crushed fruit (Heinz baby food) with added protein powder (Nature's Way) and terraria were checked twice daily for neonates.

At birth, offspring and mothers were measured (SVL ± 0.01 mm) and weighed (mass ± 0.1 mg), offspring and previously uncaught mothers toe-clipped and offspring sexed by hemipene eversion (Wapstra *et al.*, 2004, 2009). Adult females were released within 5 m of their original site of capture, whereas offspring were randomly released at one of 12 locations within the study site. Climate data were obtained from a weather station located approximately 5 km from the study site (42°34′S, 147°52′E) through the Australian Bureau of Meteorology website (Australian Government Bureau of Meteorology, 2015).

Field study: statistical analysis

Maternal body condition at the onset of vitellogenesis (C_{vit}) was used as a measure of the energy available for reproduction into the next litter prior to vitellogenesis. Each female's C_{vit} and postpartum condition (C_{pp}) were calculated as her residual mass from a regression model of mass on SVL measured immediately after parturition for all females across all years of the study (A female's C_{pp}, therefore equates to her C_{vit} in the following year). The first year of data (2000/2001), and females from subsequent years that did not give birth in the previous year were excluded when testing for the effect of C_{vit}, as no measure was available. In total, across the 16-year study, we collected data from 1395 litters across 673 unique females and were able to calculate C_{vit} for the mothers of 576 litters (Table S1). Relative litter mass (RLM), calculated as the ratio of the total litter mass to the postpartum body mass of the mother, was used to estimate reproductive effort (Shine, 1980). Cohort sex ratios presented in Fig. 1 were calculated as the number of male offspring in a year divided by the total number of offspring in that year, such that values above 0.5 indicate male-skewed cohorts and values below 0.5 indicate female-skewed cohorts.

We assessed the effect of C_{vit}, mean maximum temperature throughout the gestation period (T_{gest}; October 1 to December 31), and their interaction on mean litter offspring mass, RLM, date of birth and C_{pp} using generalized linear mixed models (LMMs), fit by REML. Maternal identity was included as a random effect, with random intercept and random slope in response to T_{gest} to account for repeated measures of mothers across years. To test whether C_{vit}, mean maximum temperature during the critical period of sex determination (T_{crit}; October 1 to November 15 (Neaves *et al.*, 2006; Wapstra *et al.*, 2009)) or their interaction had an effect on offspring sex, we fit a generalized linear mixed model (GLMM) with binomial error distribution (logit link) and maternal identity as a random effect with random intercept and random slope in response to T_{crit}, using a type II Wald χ^2 test. In all analyses, independent variables other than C_{vit} were mean-centred. C_{vit} was not centred as the mean value was very close to

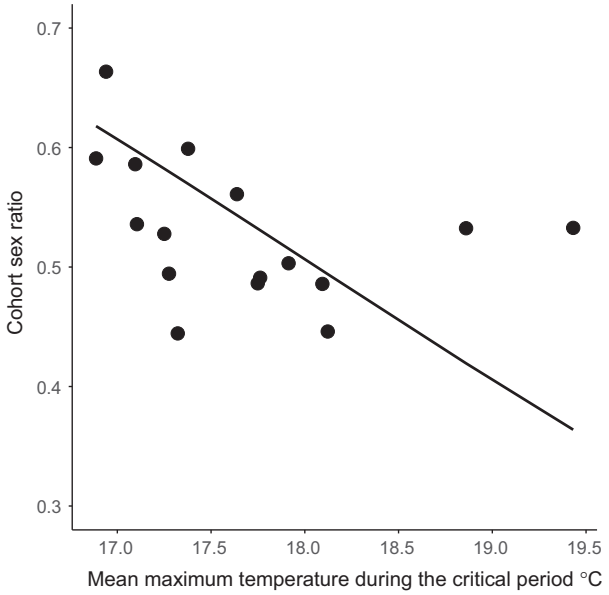


Fig. 1 Mean cohort response in *Niveoscincus ocellattus* in the field of offspring sex to temperature during the critical sex determining period (1 October to 14 November), for the seasons 2000/1 to 2015/6.

zero, only deviating from this because postpartum maternal condition for the last year of the study (2015/2016) was used to establish the relationship and because some females used to establish the relationship did not give birth in the following year. We compared the mass of male and female offspring by fitting a LMM with offspring sex as a fixed factor and with litter identity (mother within year) nested within maternal identity as random effects. The overall sex ratio was calculated for all offspring born over the duration of the study, and significance of deviation from equality was calculated using an exact binomial test.

Laboratory study: experimental procedures

One hundred and twenty-eight gravid *N. ocellatus* were collected from areas surrounding our field site in early October 2011. Females were collected, measured (SVL ± 1 mm) and weighed (± 1 mg) within the first weeks after ovulation (Wapstra *et al.*, 1999, 2009), but well prior to embryo sexual differentiation and sex determination (Neaves *et al.*, 2006; Wapstra *et al.*, 2009). Females were collected and housed using the protocol described for the field study above, with the exception that basking opportunity and food quantity were determined by random allocation to treatment groups. The model for this experiment was a 4 × 2 factorial design with four basking opportunity treatments (3-, 6-, 9- and 12-h access to the basking light) and two food quantity treatments (high and low). Basking treatments were chosen to mimic and exaggerate the yearly variation in female opportunities for basking in the field, including conditions likely to be experienced by *N. ocellatus* in extreme years (Wapstra, 2000; Wapstra *et al.*, 2004; Cadby *et al.*, 2010, 2014). More females (n = 36) were assigned to the highest (12 h) and lowest (3 h) basking treatments than to the intermediate (6 and 9 h) treatments (n = 28) to maximize the chances of detecting sex allocation responses in response to basking opportunity, which were expected to be strongest at these extremes. Half of each basking treatment group was randomly assigned to each food quantity treatment to manipulate resources available to females during gestation. Those assigned to the high-food-quantity treatment were provided with eight mealworms and 0.6 mL puree and protein powder mix at each feeding, whereas those assigned to the low-food-quantity treatment were given three mealworms and 0.3 mL puree and protein powder mix. These quantities were sufficient to provide different amounts of food while remaining within the boundaries for maintaining lizard health and condition. All lizards were provided with water *ad libitum* and were fed twice per week. Treatment groups were rotated among the four available wall sections of the laboratory, and cage positions were randomly changed within groups once per fortnight to ensure results were not confounded by position effects. Due to several females terminating pregnancies before birth or producing nonviable offspring, which could not be sexed or measured, the final sample sizes were reduced (Table S2).

Cages were checked for neonates twice per day from the 1 December. Following birth, adult females were weighed (± 1 mg), measured (SVL ± 1 mm) and litter size was recorded. Within 1 day of birth, offspring were weighed (± 0.1 mg), measured (SVL ± 0.01 mm), sexed, and given unique and permanent toe-clip identification before being released at the site from which their mothers were initially captured. At the completion of the majority of births within each treatment group, those individuals that had not yet given birth were checked for developing embryos by abdominal palpation. Nonpregnant females and females that had given birth were released at their site of capture within 1 week of birth or identification as nonpregnant.

Laboratory study: statistical analysis

Maternal condition measures were calculated using the model developed for the long-term data set. Maternal condition at ovulation (C_{ov}) was calculated from SVL and mass at capture and was mean-centred, whereas maternal postpartum condition (C_{pp}) was calculated from SVL and mass immediately after parturition. RLM and sex ratio were calculated in the same way as in the field study (see '*Field Study*' section above). The effect of the basking treatment, food quantity treatment, C_{ov} and their interactions on date of birth, mean offspring mass, RLM

and C_{pp} were assessed by fitting type II generalized linear models with basking treatment as a continuous variable (hours of basking) and food quantity treatment as a factor. To assess the effects of these variables and their interactions on sex ratio, we fit a generalized linear model (GLM) with a binomial error distribution (logit link) by a type II likelihood ratio test. Because laboratory results for the effect of temperature on offspring sex did not match those from the field study and from previous studies, we tested for an overall laboratory effect on offspring sex using an exact binomial test on the overall significance of deviation from equality of the combined sex ratio across all treatments.

All analyses were conducted in R (R Core Team, 2014) using the "lme4" package (Bates *et al.*, 2014). *P*-values, *F*-statistics and approximate denominator degrees of freedom were derived based on Kenward–Roger's approximation, using the 'lmerTest' package (Kuznetsova *et al.*, 2013). In GLMM and GLM models, type II Wald χ^2 tests and likelihood ratio tests were performed using the 'car' package (Fox & Weisberg, 2011).

Results

Field study

We found an overall sex ratio bias across years towards males; of the 3317 births recorded, 1757 were male and 1560 were female, a sex ratio of 0.53 (exact binomial test; $P < 0.001$, Table S1). There was a significant correlation between mean maximum temperature during the critical sex-determining period (T_{crit}) and offspring sex (Table 1). For each 1 °C increase in T_{crit}, the odds of offspring being male were reduced by a factor of 0.76 (Table 1, Fig. 1). In contrast, there was no significant correlation between female condition at the onset of vitellogenesis (C_{vit}) and offspring sex, nor was there a significant interactive relationship with T_{crit} (Table 1). We found strong correlations (Table 1) between the mean maximum temperature experienced during gestation (T_{gest}) and both date of birth and post-partum maternal condition (C_{pp}), and a statistically significant, but minor, correlation with reproductive effort (RLM, Table 1). Specifically, for each 1 °C increase in T_{gest}, birth occurred 5.60 ± 0.53 SE days earlier (Fig. 2a), C_{pp} decreased 0.19 ± 0.04 g (Fig. 2b), and RLM decreased 0.01 ± 0.005 SE. We found no correlation between T_{gest} and mean offspring mass (Table 1). C_{vit} had a statistically significant, but weak correlation with both RLM and C_{pp} (Table 1). For each 1 g, a mother was heavier than expected for her SVL at the beginning of vitellogenesis, RLM increased 0.018 ± 0.005 SE and C_{pp} increased 0.38 ± 0.04 SE g. There was no correlation between C_{vit} and date of birth or mean offspring mass (Table 1). Male offspring were significantly heavier than female offspring ($F_{1,2450.3} = 50.51$, $P < 0.001$), although the difference in size between the sexes was small; mean masses of males and females were 0.539 ± 0.004 SE g and 0.529 ± 0.001

Table 1 The effect of maternal condition, temperature and their interaction on litter traits in a field study of *Niveoscincus ocellatus* over the seasons 2000/01 to 2015/16.

Dependant variable	Maternal condition (C_{vit})	Temperature	Interaction
Date of birth	$F_{(1,502.99)} = 0.65$	**$F_{(1,95.66)} = 108.39$**	$F_{(1,148.69)} = 0.54$
	$P = 0.420$	**$P < 0.00001$**	$P = 0.465$
	$\beta = -0.460 \pm 0.566$ SE	**$\beta = -5.597 \pm 0.527$ SE**	$\beta = -0.761 \pm 1.017$ SE
Mean offspring mass	$F_{(1,551.99)} = 1.96$	$F_{(1,96.55)} = 2.20$	$F_{(1,145.95)} = 0.19$
	$P = 0.162$	$P = 0.142$	$P = 0.663$
	$\beta = 0.005 \pm 0.004$	$\beta = 0.005 \pm 0.004$	$\beta = -0.003 \pm 0.007$
Offspring sex	$\chi^2 = 0.004$	**$\chi^2 = 11.236$**	$\chi^2 = 0.013$
	$P = 0.951$	**$P < 0.001$**	$P = 0.908$
	$\beta = -0.005 \pm 0.053$ SE	**$\beta = -0.273 \pm 0.081$ SE**	$\beta = 0.016 \pm 0.136$ SE
Relative litter mass (RLM)	**$F_{(1,547.23)} = 11.08$**	**$F_{(1,100.87)} = 5.92$**	$F_{(1,153.23)} < 0.001$
	$P < 0.001$	**$P = 0.017$**	$P = 0.990$
	$\beta = 0.018 \pm 0.005$ SE	**$\beta = 0.013 \pm 0.005$ SE**	$\beta = 0.0001 \pm 0.010$ SE
Postpartum maternal condition (C_{pp})	**$F_{(1,514.44)} = 101.50$**	**$F_{(1,92.72)} = 25.72$**	$F_{(1,133.33)} = 0.20$
	$P < 0.0001$	**$P < 0.0001$**	$P = 0.654$
	$\beta = 0.376 \pm 0.037$ SE	**$\beta = -0.187 \pm 0.036$ SE**	$\beta = -0.033 \pm 0.071$ SE

Fixed effect estimates are from LMM and GLMM models. *P*-values, *F*-statistics, χ^2 and β values for main effects shown are from models excluding interactions. Significant results are indicated in bold. Temperature throughout gestation (T_{gest}) is used in all models except where offspring sex is the response variable, in which case temperature during the critical sex determination period (T_{crit}) is used instead. Maternal condition (C_{vit}) was calculated following birth the previous year (i.e. at the beginning of vitellogenesis). β values indicate the change in the response variable for each unit change in the independent variable except where the response is offspring sex, in which case β is the log factor change to the odds of male: female offspring for each unit change in the independent variable.

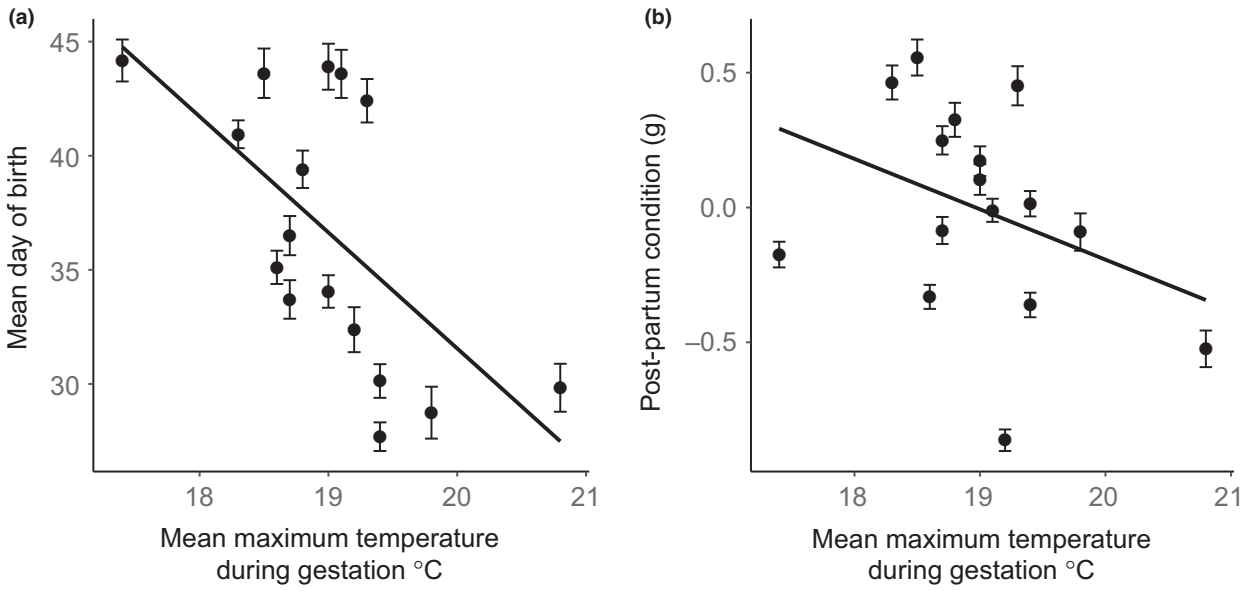


Fig. 2 Mean cohort response in *Niveoscincus ocellattus* in the field of (a) date of birth and (b) postpartum maternal condition (C_{pp}) in response to temperature experienced during the gestation period (1 October to 31 December) for the seasons 2000/1 to 2015/6.

SE g, respectively. There was no interaction between T_{gest} and C_{vit} on any of the measured variables (Table 1).

Experimental study

The overall sex ratio in the experimental study was male-skewed; of the 204 births recorded, 128 were males and 76 were female (exact binomial test; $P = 0.001$; Table 2). We found no independent or interactive effects of basking treatment, food quantity treatment or maternal condition at ovulation (C_{ov}) on offspring sex or RLM (Table 3); although sex ratios showed a slight increase with increasing basking opportunity (Table 2), this effect was nonsignificant (Table 3). Basking treatment had a significant effect on date of birth and mean offspring mass but no significant effect on postpartum condition (Table 3). Females given more opportunity to bask produced earlier litters (-6.881 ± 0.460 SE days per hour of basking; Table 2, Fig. S1a) consisting of larger offspring ($+ 0.008 \pm 0.002$ SE g per hour of basking; Table 3, Fig. S1b) than females given less access to basking. Food quantity treatment had a marginally significant effect on C_{pp} and had no significant effect on date of birth or mean offspring mass (Table 3). Females in the low-food-quantity treatment were in worse condition after giving birth (-0.276 ± 133 SE g) than those in the high-food-quantity treatment (Table 2). C_{ov} had a significant effect on C_{pp} and a marginally significant effect on mean offspring mass (Table 3). For each increase in C_{ov} of 1 g, females' C_{pp} increased 0.576 ± 0.143 SE g and mean offspring mass increased 0.027 ± 0.013 SE g (Table 2).

Discussion

Differential sex allocation is expected when intrinsic (e.g. body condition) or extrinsic (e.g. temperature, resource availability) factors influence variation in traits that differentially affect the fitness of male and female offspring (Charnov, 1982; Uller & Olsson, 2006; Uller *et al.*, 2007; Wapstra & Warner, 2010), or when the cost of producing offspring varies between offspring of different sexes (Fisher, 1930; Charnov, 1982). While most studies consider only a single causal factor, patterns may be obscured if multiple factors influence sex allocation simultaneously. Here, we examined possible interactive and independent effects of temperature and resource availability on sex allocation in *N. ocellatus*.

We found evidence that yearly variation in sex ratios is strongly linked to annual variation in temperature during embryo development in a natural population, with the proportion of female offspring increasing in warmer years, and of male offspring in cooler years. These patterns are in accordance with our previous related work (see Table S3) in this system showing strong temperature effects on offspring sex ratios in the field (Wapstra *et al.*, 2004, 2009; Pen *et al.*, 2010; Cunningham *et al.*, 2017), and in the laboratory (Wapstra *et al.*, 2004; Pen *et al.*, 2010). Indeed, we have explicitly modelled the evolution of TSD in this population (Pen *et al.*, 2010), which explained the temperature effect as resulting from sex-specific fitness effects of date of birth, which was affected by thermal environment in both our field and laboratory studies.

In contrast, we found no evidence that female condition or resource availability at any stage during the

Table 2 Sample sizes, maternal characteristics and litter characteristics of *Niveoscincus ocellatus* across treatment groups (Basking opportunity and nutrition) in a laboratory experiment. Values are means ± standard errors.

Basking treatment (*B*)	3-h		6-h		9-h		12-h		Combined basking treatments	
Food quantity treatment (Q)	Low	High	Low	High	Low	High	Low	High	Low	High
Pretreatment sample size	18	18	14	14	14	14	18	18	64	64
Final sample size	8	8	11	12	10	11	13	15	42	46
	16		23		21		28		88	
Condition at ovulation (C_{ov})	−0.07 ± 0.10	−0.37 ± 0.15	−0.46 ± 0.13	−0.32 ± 0.14	−0.07 ± 0.18	−0.17 ± 0.14	−0.24 ± 0.11	−0.19 ± 0.15	−0.22 ± 0.07	−0.25 ± 0.07
	−0.22 ± 0.10		−0.39 ± 0.09		−0.12 ± 0.11		−0.21 ± 0.09		−0.24 ± 0.05	
Date of birth	93.5 ± 6.1	90.9 ± 8.1	63.9 ± 4.6	63.1 ± 3.6	35.3 ± 4.4	40.5 ± 2.7	30.3 ± 2.9	27.5 ± 2.3	52.33 ± 4.3	50.9 ± 3.9
	92.2 ± 4.9		63.5 ± 2.8		38.0 ± 2.5		28.8 ± 1.8		51.6 ± 2.9	
Mean offspring mass (g)	0.48 ± 0.02	0.47 ± 0.01	0.49 ± 0.02	0.52 ± 0.02	0.54 ± 0.02	0.55 ± 0.02	0.54 ± 0.01	0.56 ± 0.02	0.52 ± 0.01	0.53 ± 0.01
	0.48 ± 0.01		0.51 ± 0.01		0.55 ± 0.01		0.55 ± 0.01		0.52 ± 0.01	
Total offspring (males)	16 (9)	20 (15)	28 (18)	32 (20)	25 (16)	25 (15)	24 (15)	34 (20)	93 (58)	111 (70)
	36 (24)		60 (38)		50 (31)		58 (35)		204 (128)	
Sex ratio	0.563	0.750	0.643	0.625	0.640	0.600	0.625	0.588	0.624	0.631
	0.667		0.633		0.620		0.603		0.627	
Relative litter mass (RLM)	0.19 ± 0.02	0.24 ± 0.02	0.27 ± 0.03	0.26 ± 0.01	0.22 ± 0.04	0.22 ± 0.01	0.21 ± 0.02	0.23 ± 0.02	0.22 ± 0.01	0.24 ± 0.01
	0.22 ± 0.02		0.27 ± 0.01		0.22 ± 0.02		0.22 ± 0.01		0.23 ± 0.01	
Post-partum condition (C_{pp})	0.42 ± 0.31	0.52 ± 0.19	0.16 ± 0.15	0.58 ± 0.24	0.53 ± 0.10	0.57 ± 0.25	0.03 ± 0.22	0.42 ± 0.16	0.26 ± 0.10	0.52 ± 0.10
	0.47 ± 0.17		0.38 ± 0.15		0.55 ± 0.13		0.24 ± 0.13		0.39 ± 0.07	

reproductive cycle influenced sex allocation either in interaction with temperature or independently. There are several potential explanations for this lack of effects, which are related to our underlying assumptions for how resource availability might influence sex-specific fitness benefits of producing males vs. females. We argued that resource availability could influence offspring size at birth (*sensu* generalized Trivers & Willard, 1973), which could in turn influence offspring size at maturity, and that this may have sex-specific effects on fitness (Pen *et al.*, 2010). For this to occur, however, two conditions would have to be met. First, females in good condition, or with increased access to resources, would have to divert those additional resources to their offspring rather than to their own condition or growth. We found limited support for this; although females in better condition at the onset of vitellogenesis did increase their reproductive effort in our field study, this effect was minor and was not replicated in the laboratory. Rather, we found that females in better condition (at both the onset of vitellogenesis, and at ovulation) and those provided with more food during gestation were in better condition after giving birth. This suggests that there may be selection on females to devote additional resources towards increasing their own condition and growth rather than towards increasing offspring size (Itonaga *et al.*, 2012a). Second, there would have to be a sex-specific link between size at birth and fitness. We have previously shown that date of birth has strong effects on offspring growth before winter and ultimately adult body size in this system and that this has resulted in strong selection for the coupling of temperature and sex determination (Pen *et al.*, 2010). For size at birth to have a similar effect, it would have to predictably translate to a larger size at the end of the activity season. Post-partum growth in *N. ocellatus* is rapid and effects influencing post-partum growth (e.g. Itonaga *et al.*, 2012a,b; Cadby *et al.*, 2014) might offset the initial small differences in size at birth. Thus, size at birth might be a poor predictor of later size (Qualls & Shine, 2000). Alternatively, resource availability could influence sex allocation indirectly if it affected maternal basking behaviour and therefore affected date of birth. However, we found no effect of available resources, either alone or in interaction with thermal environment, on date of birth. The absence of an effect of resource availability on date of birth suggests that pregnant females do not alter their basking decisions according to their condition. Combined, these results suggest that there is little evidence for the effects of resource availability on the key life history traits that might provide a mechanistic link between the environment and maternal sex allocation decisions in *N. ocellatus*. Thus, selection to couple sex to resource availability may be relatively weak in this system.

One component of the study that requires additional discussion was the discordance between the strong

Table 3 The effect of basking treatment (B), food quantity treatment (Q), maternal condition at ovulation (C_{ov}) and their interactions on litter characteristics and post-partum condition (C_{pp}) of *Niveoscincus ocellatus* females in a laboratory experiment.

Dependant Variable	Basking treatment (B)	Food quantity treatment (Q)	Maternal condition (C_{ov})	B*Q interaction	B*C_{ov} interaction	Q*C_{ov} interaction	B*Q*C_{ov} interaction
Date of birth	**$F_{(1,84)}$ = 226.64 P < 0.00001**	$F_{(1,84)}$ = 0.23 P = 0.636	$F_{(1,84)}$ = 0.45 P = 0.504	$F_{(1,81)}$ = 0.03 P = 0.861	$F_{(1,81)}$ = 0.14 P = 0.710	$F_{(1,81)}$ = 2.37 P = 0.128	$F_{(1,80)}$ = 2.99 P = 0.087
Mean offspring mass	**$F_{(1,84)}$ = 17.83 P < 0.0001**	$F_{(1,84)}$ = 1.59 P = 0.210	**$F_{(1,84)}$ = 4.07 P = 0.05**	$F_{(1,81)}$ = 0.13 P = 0.719	$F_{(1,81)}$ = 2.01 P = 0.160	$F_{(1,81)}$ = 2.09 P = 0.152	$F_{(1,80)}$ = 0.98 P = 0.325
Offspring sex	χ^2 = 0.331 P = 0.565	χ^2 = 0.0002 P = 0.988	χ^2 = 0.007 P = 0.933	χ^2 = 0.935 P = 0.331	χ^2 = 0.579 P = 0.447	χ^2 = 0.507 P = 0.477	χ^2 = 1.526 P = 0.217
Relative litter mass (RLM)	$F_{(1,84)}$ = 0.45 P = 0.504	$F_{(1,84)}$ = 0.78 P = 0.381	$F_{(1,84)}$ = 0.58 P = 0.448	$F_{(1,81)}$ = 0.06 P = 0.813	$F_{(1,81)}$ = 0.01 P = 0.917	$F_{(1,81)}$ = 0.30 P = 0.584	$F_{(1,80)}$ = 0.04 P = 0.844
Postpartum maternal condition (C_{pp})	$F_{(1,84)}$ = 0.97 P = 0.327	**$F_{(1,84)}$ = 3.82 P = 0.054**	**$F_{(1,84)}$ = 16.12 P < 0.001**	$F_{(1,81)}$ = 0.002 P = 0.963	$F_{(1,81)}$ = 0.001 P = 0.972	$F_{(1,81)}$ = 0.04 P = 0.839	$F_{(1,80)}$ = 0.20 P = 0.659

Fixed effect estimates are from LMM and GLMM models. P-values, F-statistics and χ^2 values shown are from reduced models excluding higher order interactions. Significant results are indicated in bold. Model coefficients for main effects are presented in Table S2. Maternal condition (C_{ov}) was calculated at ovulation.

effects of temperature on sex allocation in the field and the lack of effects of temperature observed in the laboratory, where the correlation between increasingly male-skewed litters and decreasing basking opportunity (i.e. maternal body temperature) was nonsignificant and sex ratios were male-skewed in all basking treatments. Discordant effects in replicated sex ratio studies are not uncommon, especially in laboratory experiments (see, e.g., Parker, 2012; Booksmythe *et al.*, 2017). There are several possible explanations for our results. First, the birth dates invoked by our laboratory study were outside the range of birth dates that we have recorded across the 17-year period of our field study. Specifically, in our 12-h basking treatment, the mean date of birth was earlier than all but one of those observed across the 17 years of field work, whereas birth dates in the 6- and 3-h treatments were later than any observed over this period, corresponding to field temperatures throughout gestation of approximately 20.5 °C, 14.3 °C and 9.2 °C, respectively (see Fig. 2a and Fig. S1a). Thus, thermal conditions in these treatments sit beyond the range of temperatures across which we have modelled the reaction norm of offspring sex response to temperature in this species, and beyond the range of temperatures they are exposed to in the wild. As thermal reaction norms with respect to temperature can have a variety of forms (e.g. MF, FM, FMF, MFM), it is possible that what we are observing is a species with a U-shaped, as opposed to a linear, reaction norm (i.e. an MFM pattern, in which males are common at both high and low temperatures and females at intermediate temperatures; e.g. Luckenbach *et al.*, 2009 in flatfishes), although this pattern has not been reported in reptiles (Quinn *et al.*, 2011). While further research is required to confirm this pattern of response, it would be consistent with the increase in male-biased litters observed in the two warmest years in our field population (Fig. 1). Second, these results could have been a result of a laboratory artefact, whereby consequences of animals being kept in the laboratory (e.g. altered hormone profiles) may have impacted sex determination and thus masked any effect of temperature. For instance, laboratory-induced stress may have elevated levels of circulating corticosterone, which has been shown to have significant effects on sex determination in other reptile species (e.g. Warner *et al.*, 2009). Finally, the relatively shallow reaction norm between temperature and offspring sex in this species and the variation observed in the wild may have meant that we did not have the power to tease out the relatively subtle effects of temperature in our laboratory experiment.

In summary, our results suggest that incorporating resource availability into our models of sex allocation decisions in *N. ocellatus* does not add additional explanatory power over and above considering temperature alone. It is, however, possible that other factors not tested in this study such as stress, age, mate quality, population dynamics or resource availability across other temporal scales may influence sex allocation decisions in this system. For example, factors from previous years may influence reproductive effort in the current year (e.g. Doughty & Shine, 1998; Bleu *et al.*, 2013) or effects may vary at different stages of the reproductive cycle. Nevertheless, none of female body condition at the beginning of vitellogenesis or at ovulation, food availability during gestation (this study), nor mating history (While & Wapstra, 2009) have been shown to be important for predicting patterns of sex allocation in *N. ocellatus.* It is therefore plausible that temperature is the sole factor influencing sex allocation in this species.

Acknowledgments

We thank Jo McEvoy, Mandy Caldwell and Kirke Munch for assistance with fieldwork, husbandry and

processing. We would also like to thank Adam McKiernan, Moe McGruber and Erin Taylor for their support.

Author contributions

All authors contributed to the collection and compilation of field data, J.G. performed the laboratory experiment and G.D.C. performed the analysis. All authors wrote the manuscript and agreed to be accountable for all aspects of the research.

Funding

This research was supported by an ARC Future Fellowship (FT110100597 awarded to E.W.) and an ARC DECRA Fellowship (DE150100336 awarded to G.M.W.). G.D.C. was supported by the Holsworth Wildlife Research Fund. E.W. was supported by the Foundation of National Parks.

Ethics

All work was carried out with approval from the Animal Ethics Committee at the University of Tasmania (Ethics Approval number A0012087).

References

Bates, D., Maechler, M., Bolker, B. & Walker, S. 2014. *lme4: Linear mixed-effects models using Eigen and S4*. R package version 1.1-7, http://CRAN.R-project.org/package=lme4.

Bleu, J., Le Galliard, J.-F., Fitze, P., Meylan, S., Clobert, J. & Massot, M. 2013. Reproductive allocation strategies: a long-term study on proximate factors and temporal adjustments in a viviparous lizard. *Oecologia* **171**: 141–151.

Booksmythe, I., Mautz, B., Davis, J., Nakagawa, S. & Jennions, M.D. 2017. Facultative adjustment of the offspring sex ratio and male attractiveness: a systematic review and meta-analysis. *Biol. Rev.* **92**: 108–134.

Cadby, C.D., While, G.M., Hobday, A.J., Uller, T. & Wapstra, E. 2010. Multi-scale approach to understanding climate effects on offspring size at birth and date of birth in a reptile. *Integr. Zool.* **5**: 164–175.

Cadby, C.D., Jones, S.M. & Wapstra, E. 2011. Potentially adaptive effects of maternal nutrition during gestation on offspring phenotype of a viviparous reptile. *J. Exp. Biol.* **214**: 4234–4239.

Cadby, C.D., Jones, S.M. & Wapstra, E. 2014. Geographical differences in maternal basking behaviour and offspring growth rate in a climatically widespread viviparous reptile. *J. Exp. Biol.* **217**: 1175–1179.

Cameron, E.Z. & Dalerum, F. 2009. A Trivers-Willard effect in contemporary humans: male-biased sex ratios among billionaires. *PLoS ONE* **4**: e4195.

Charnov, E.L. 1979. Genetic evolution of patterns of sexuality – Darwinian fitness. *Am. Nat.* **113**: 465–480.

Charnov, E.L. 1982. *The Theory of Sex Allocation*. Princeton University Press, Princeton, NJ.

Cockburn, A., Legge, S. & Double, M.C. 2002. Sex ratio in birds and mammals: can the hypotheses be disentangled? In: *Sex Ratios Concepts and Research Methods* (I.C.W. Hardy, ed), pp. 266–286. Cambridge University Press, Cambridge.

Conover, D.O. 1984. Adaptive significance of temperature-dependent sex determination in a fish. *Am. Nat.* **123**: 297–313.

Cunningham, G.D., While, G.M. & Wapstra, E. 2017. Climate and sex ratio variation in a viviparous lizard. *Biol. Lett.* **13**: 20170218.

Doughty, P. & Shine, R. 1998. Reproductive energy allocation and long-term energy stores in a viviparous lizard (*Eulamprus tympanum*). *Ecology* **79**: 1073–1083.

Fisher, R.A. 1930. *The Genetical Theory of Natural Selection*. Oxford University Press, Oxford.

Fox, J. & Weisberg, S. 2011. *An R Companion to Applied Regression*, 2nd edn. Sage, Thousand Oaks CA. http://socserv.socsci.mcmaster.ca/jfox/Books/Companion

Frank, S.A. 1990. Sex allocation theory for birds and mammals. *Annu. Rev. Ecol. Syst.* **21**: 13–55.

Itonaga, K., Jones, S.M. & Wapstra, E. 2012a. Do gravid females become selfish? Female allocation of energy during gestation. *Physiol. Biochem. Zool.* **85**: 231–242.

Itonaga, K., Jones, S.M. & Wapstra, E. 2012b. Effects of maternal basking and food quantity during gestation provide evidence for the selective advantage of matrotrophy in a viviparous lizard. *PLoS ONE* **7**: e41835.

Komdeur, J. 1996. Facultative sex ratio bias in the offspring of Seychelles warblers. *Proc. R. Soc. Lond. B Biol. Sci.* **263**: 661–666.

Komdeur, J. & Pen, I. 2002. Adaptive sex allocation in birds: the complexities of linking theory and practice. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **357**: 373–380.

Kuznetsova, A., Brockhoff, P.B. & Christensen, R.H.B. 2013. *lmerTest: Tests for random and fixed effects for linear mixed effect models (lmer objects of lme4 package)*. http://CRAN.R-project.org/package=lmerTest

Luckenbach, J.A., Borski, R.J., Daniels, H.V. & Godwin, J. 2009. Sex determination in flatfishes: mechanisms and environmental influences. *Semin. Cell Dev. Biol.* **20**: 256–263.

Neaves, L., Wapstra, E., Birch, D., Girling, J.E. & Joss, J.M.P. 2006. Embryonic gonadal and sexual organ development in a small viviparous skink, *Niveoscincus ocellatus*. *J. Exp. Zoolog. Part A Comp. Exp. Biol.* **305A**: 74–82.

Olsson, M. & Shine, R. 2001. Facultative sex allocation in snow skink lizards (*Niveoscincus microlepidotus*). *J. Exp. Biol.* **14**: 120–128.

Parker, T.H. 2012. What do we really know about the signalling role of plumage colour in blue tits? A case study of impediments to progress in evolutionary biology. *Biol. Rev.* **88**: 511–536.

Pen, I., Uller, T., Feldmeyer, B., Harts, A., While, G.M. & Wapstra, E. 2010. Climate-driven population divergence in sex-determining systems. *Nature* **468**: 436–438.

Qualls, F.J. & Shine, R. 2000. Post-hatching environment contributes greatly to phenotypic variation between two populations of the Australian garden skink, *Lampropholis guichenoti*. *Biol. J. Linn. Soc.* **71**: 315–341.

Quinn, A.E., Sarre, S.D., Ezaz, T., Marshall Graves, J.A. & Georges, A. 2011. Evolutionary transitions between mechanisms of sex determination in vertebrates. *Biol. Lett.* **7**: 443–448.

R Core Team. 2014. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Radder, R.S., Pike, D.A., Quinn, A.E. & Shine, R. 2009. Offspring sex in a lizard depends on egg size. *Curr. Biol.* **19**: 1102–1105.

Seger, J. & Stubblefield, J.W. 2002. Models of sex ratio evolution. In: *Sex Ratios Concepts and Research Methods* (I.C.W. Hardy, ed), pp. 2–25. Cambridge University Press, Cambridge.

Shine, R. 1980. Costs of reproduction in reptiles. *Oecologia* **46**: 92–100.

Thompson, M.B., Speake, B.K., Stewart, J.R., Russell, K.J. & McCartney, R.J. 2001. Placental nutrition in the Tasmanian skink, *Niveoscincus ocellatus*. *J. Comp. Physiol. B.* **171**: 155–160.

Trivers, R.L. & Willard, D.E. 1973. Natural selection of parental ability to vary the sex-ratio of offspring. *Science* **179**: 90–92.

Uller, T. & Olsson, M. 2006. No seasonal sex-ratio shift despite sex-specific fitness returns of hatching date in a lizard with genotypic sex determination. *Evolution* **60**: 2131–2136.

Uller, T. & While, G.M. 2014. Evaluation of offspring size-number invariants in 12 species of lizard. In: *Reproductive Biology and Phylogeny of Lizards and Tuatara* (J.L. Rheubert, D.S. Siegel, S.E. Trauth, eds), pp. 425–447. CRC Press, Boca Raton, FL.

Uller, T., Pen, I., Wapstra, E., Beukeboom, L.W. & Komdeur, J. 2007. The evolution of sex ratios and sex-determining systems. *Trends Ecol. Evol.* **22**: 292–297.

Uller, T., While, G.M., Cadby, C.D., Harts, A., O'Connor, K., Pen, I. *et al.* 2011. Altitudinal divergence in maternal thermoregulatory behaviour may be driven by differences in selection on offspring survival in a viviparous lizard. *Evolution* **65**: 2313–2324.

Wapstra, E. 2000. Maternal basking opportunity affects juvenile phenotype in a viviparous lizard. *Funct. Ecol.* **14**: 345–352.

Wapstra, E. & O'Reilly, J.M. 2001. Potential 'costs of reproduction' in a skink: inter- and intrapopulational variation. *Austral Ecol.* **26**: 179–186.

Wapstra, E. & Swain, R. 2001. Geographic and annual variation in life-history traits in a temperate zone Australian skink. *J. Herpetol.* **35**: 194–203.

Wapstra, E. & Warner, D.A. 2010. Sex allocation and sex determination in squamate reptiles. *Sex. Dev.* **4**: 110–118.

Wapstra, E., Swain, R., Jones, S.M. & O'Reilly, J. 1999. Geographic and annual variation in reproductive cycles in the Tasmanian spotted snow skink, *Niveoscincus ocellatus* (Squamata: Scincidae). *Aust. J. Zool.* **47**: 539–550.

Wapstra, E., Swain, R. & O'Reilly, J.M. 2001. Geographic variation in age and size at maturity in a small Australian viviparous skink. *Copeia* **3**: 646–655.

Wapstra, E., Olsson, M., Shine, R., Edwards, A., Swain, R. & Joss, J.M.P. 2004. Maternal basking behaviour determines offspring sex in a viviparous reptile. *Proc. R. Soc. Lond. B Biol. Sci.* **271**: S230–S232.

Wapstra, E., Uller, T., Sinn, D.L., Olsson, M., Mazurek, K., Joss, J. *et al.* 2009. Climate effects on offspring sex ratio in a viviparous lizard. *J. Anim. Ecol.* **78**: 84–90.

Wapstra, E., Uller, T., While, G.M., Olsson, M. & Shine, R. 2010. Giving offspring a head start in life: field and experimental evidence for selection on maternal basking behaviour in lizards. *J. Evol. Biol.* **23**: 651–657.

Warner, D.A. & Shine, R. 2008. The adaptive significance of temperature-dependent sex determination in a reptile. *Nature* **451**: 566–568.

Warner, D.A., Radder, R.S. & Shine, R. 2009. Exposure during embryonic development affects offspring growth and sex ratios in opposing directions in two lizard species with environmental sex determination. *Physiol. Biochem. Zool.* **82**: 363–371.

West, S.A. 2009. *Sex Allocation*. Princeton University Press, Oxford.

While, G.M. & Wapstra, E. 2009. Snow skinks (*Niveoscinus ocellatus*) do not shift their sex allocation patterns in response to mating history. *Behaviour* **146**: 1405–1422.

Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1 Effect of basking treatment on (a) date of birth and (b) mean offspring mass in *Niveoscincus ocellatus* in a laboratory experiment.

Table S1 Data collected in a field study across the seasons 2000/1 to 2015/6.

Table S2 Main effect model coefficients from generalised (logit) linear model (Sex ratio) and general linear models (remaining variables) in a 4 × 2 factorial laboratory experiment with four basking treatments (3, 6, 9 and 12-hours) and two food quantity treatments (high and low) in *Niveoscincus ocellatus*.

Table S3 Summary of previously reported studies demonstrating a relationship between environmental temperature during the period of sex determination and offspring sex and between date of birth and offspring sex.

Received 26 July 2017; revised 26 November 2017; accepted 27 November 2017